BLAST 2 SEQUENCES

This tool produces the alignment of two given sequences using BLAST engine for local alignment.
The stand-alone executable for blasting two sequences (bl2seq) can be retrieved from NCBI ftp site
Reference: Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250

Program blastp Matrix BLOSUM62

Parameters used in BLASTN program only:
Reward f r a match: **Penalty for a mismatch:**

Use Mega BLAST Strand option Not Applicable

Open gap 11 and extension gap 1 penalties
gap x_dropoff 50 expect 10.0 word size 3 Filter Align

Sequence 1 Enter accession or GI SEQ 4 or download from file
or sequence in FASTA format from: 0 to: 0

```
qsegpavvniqaapaprtqngsgnaetasapiaasapryerikrivpnmpeipqeeaaqgg
lnfgsgfiisknqyiltnthvvagmgsikvllndkreytakligsdvqsdvallkidatee
lpvvkignpknlkpgewvaaigapfgfdnsvtagivsakgrslpnesytpfiqtdvainpg
nsggplfnlkgqvvginsqiysrsggfmgisfaipidvamnvaeqlkntgkvqrgqlgvii
qevsyglaqsfgldkasgaliakilpgspaeraglqagdivlsldggeirssgdlpvmvga
itpgkevslgvwrkgeeitikaklgnaaehtgassktdeapyteqqsgtfsvesagitlqt
htdssgkhlvvvrvsdaaeraglrhgdeilavrasprq
```

Sequence 2 Enter accession or GI Gilbert4 or download from file
or sequence in FASTA format from: 0 to: 0

```
mgikkvcitvicilvicigiyticarvnqgernavsiikakifneegkpvnlifcytiqm
kvaerimaqhpgerfyvvlmsenrnekydyyfnqikdkaerayffylpyglnksfnfiptm
aelkvksmllpkvkriylaslekvsiaaflstypdaeiktfddgtnnliressylggefav
ngaikrnfarmmvgdwsiaktrnasdehytifkglknimddgrrkmtylplfdaselkagd
etggtvrillgspdkemkeisekaaknfniqyvaphprqtyglsgvtalnspyviedyilr
eikknphtryeiytffsgaaltmkdfpnvhvyalkpaslpedywlkpvyalfrqadipilt
fddkn
```

Align Clear Input

Comments and suggestions to *blast-help@ncbi.nlm.nih.gov*

Blast 2 Sequences results

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4 [Aug-26-2002]

Matrix BLOSUM62 gap open: 11 gap extension: 1
x_dropoff: 50 expect: 10.0 wordsize: 3 Filter ☑ Align

Sequence 1 lcl|seq_1 **Length** 465 09/839,090 SEQ ID NO: 4

Sequence 2 lcl|seq_2 **Length** 371 (c.f. 09/6,529 SEQ ID NO: 2

N significant similarity was found

BLAST 2 SEQUENCES

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Program blastp Matrix BLOSUM62

Parameters used in BLASTN program only:
Reward for a match: **Penalty for a mismatch:**

☐ Use Mega BLAST Strand option Not Applicable

Open gap 11 and extension gap 1 penalties
gap x_dropoff 50 expect 10.0 word size 3 Filter ☑ Align

Sequence 1 Enter accession or GI SEQ 4 or download from file
or sequence in FASTA format from: 0 to: 0

```
qsegpavvniqaapaprtqngsgnaetasapiaasapryerrkrivpnmpeipqeeaaagg
lnfgsgfiiskngyiltnthvvagmgsikvllndkreytakligsdvqsdvallkidatee
lpvvkignpknlkpgewvaaigapfgfdnsvtagivsakgrslpnesytpfiqtdvainpg
nsggplfnlkgqvvginsqiysrsggfmgisfaipidvamnvaeqlkntgkvqrgqlgvii
qevsyglaqsfgldkasgaliakilpgspaeraglqagdivlsldggeirssgdlpvmvga
itpgkevslgvwrkgeeitikaklgnaaehtgassktdeapyteqqsgtfsvesagitlqt
htdssgkhlvvvrvsdaaeraglrhgdeilavrasprq
```

Sequence 2 Enter accession or GI Gilbert2 or download from file
or sequence in FASTA format from: 0 to: 0

```
mgikkacitvicilvrcrgiryticrvnngernavsiikqkirneegepvnilicytiiqm
kvaerimaqhpgerfyvvlmsenrnekydyyfkqikdkaerayffhlpyglnksfnfiptm
aelkvksmllpkvkriylaslekvsiaaflstypdaeiktfddgtgnliqsssylgdefsv
ngtikrnfarmmigdwsiaktrnasdehytifkglknimddgrrkmtylplfdaselkagd
etggtvrillgspdkemkeisekaaknfniqyvaphprqtyglsgvttlnspyviedyilr
eikknphtryeiytffsgaaltmkdfpnvhvyalkpaslpedywlkpvyalftqsgipilt
fddkn
```

Align Clear Input

Comments and suggestions to *blast-help@ncbi.nlm.nih.gov*

Blast 2 Sequences results

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.4 [Aug-26-2002]

Matrix: BLOSUM62 gap open: 11 gap extension: 1
x_dropoff: 50 expect: 10.0 wordsize: 3 Filter: ☑ Align

Sequence 1 lcl|seq_1 **Length** 465 09/358,090 SEQ ID NO:4

Sequence 2 lcl|seq_2 **Length** 371 6,096,529 SEQ ID NO: 4

No significant similarity was found

Express Mail No.: EL 500 578 282 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Jackson and Harris

Serial No.: 09/388,090 Art Unit: 1645

Filed: August 31, 1999 Examiner: S. Devi

For: NEISSERIA SPP. POLYPEPTIDE, NUCLEIC ACID SEQUENCE AND USES THEREOF

Attorney Docket No.: 7969-082-999

Declaration of Dr. W. James Jackson Under C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

I, Dr. W. James Jackson, declare and state:

1. I am a co-inventor of the above-identified application which I have read and understand.

2. The following experiments were conducted under my supervision and control. The results obtained are shown in Table 2 below. As detailed below, the results obtained clearly demonstrate that the isolated *Neisseria* polypeptide designated "NGSP" polypeptide taught in the application is useful to induce an immune response against infection by *Neisseria gonorrhea*.

3. Methods: The murine model of vaginal colonization was used to evaluate the ability of the isolated NGSP polypeptide of the invention, as described in Jerse, 1999, Infec. Immun. 67(11): 5699-5708, a copy of which is attached as Exhibit A, as modified as detailed below.

3a. The NGSP polypeptide (containing a His tag) was recombinantly expressed in *E. coli* JM109 containing plasmid pTLZ-NgHtr A#2 (ATCC number PTA-470) (see

Sections 8.3 and 8.4 of the application) and isolated using affinity chromotograph as described in the application in Section 5.3.

3b. Balb/c female mice, six to ten weeks of age, were immunized three times at two week intervals with isolated recombinant NGSP polypeptide. Animals were immunized, parenterally (s.c.) according to the groupings defined in Table 1 below.

3c. Via parenteral immunization, the NGSP polypeptide was coadministered with Freund's complete adjuvant (CFA) at the first immunization. An equal volume of CFA was mixed with the NGSP polypeptide antigen solution to form an emulsion. At the second and third immunizations, Freund's incomplete adjuvant (IFA) was co-administered with the NGSP polypeptide. An equal volume of IFA was mixed with the NGSP polypeptide antigen solution to form an emulsion. Animals immunized three times with PBS alone served as negative controls, while animals immunized with formalin treated *Neisseria gonorrhea* whole cell antigen served as positive controls.

Table 1

Group Number	Antigen Amount per Dose[1]	Adjuvant
1	NGSP polypeptide (50)	CFA/IFA
2 positive control	Inactivated *Neisseria gonnorrhea* whole cell antigen	CFA/IFA
3 negative control	PBS Only	None

1 Antigen dose is given in micrograms (μg).

3d. Retroorbital bleeds were taken prior to the first immunization and again approximately ten days following the third and final dose. Approximately ten days after the last immunizatoin, a slow release pellet of β-estradiol (5.0mg released over 21 days; Innovative Research Inc.) was implanted under the skin of each animal to stabilize and synchronize the uterine cycle prior to and during the challenge phase of the experiment. Animals were challenged three to five days later by instilling into the vaginal canal a 20 μl volume of PBS containing ~1.0 X 10^6 cfu of the *N. gonorrhea* strain MS11A.

3c. For challenge, *N. gonnorhea* strain MS11A was grown from frozen stock cultures on Thayer Martin chocolate agar plates, collected by swabbing the sterile applicators and suspended in PBS. The *N. gonnorhea* strain MS11A suspension was diluted with PBS to ~1.0 X 10^8 cfu/ml using a previously generated $O.D._{600}$ vs cfu standard curve.

3f. After challenge (48hrs post challenge) the level of *N. gonnorhea* vaginal colonization was assessed by swabbing the vaginal canal with pediatric nasopharengyl Dacron/Polyester swabs. Swabs were inserted gently into the canal until resistance was encountered then gently rotated (10 complete 360° turns) to collect infectious microorganisms. Swabs were removed, moistened by brief immersion into sterile PBS and used to streak plate Thayer Martin chocolate agar plates containing antibiotics (vancomycin, colistin, nystatin, timethoprim). A standard quantitative dilution streak plating method was used to enumerate colonies. Plates were incubated at 37°C under oxygen depleted or microaerophilic conditions for 24 to 72 hours prior to counting.

Protective efficacy is expressed as percent of animals protected in a vaccination group.

4. Results: Results are presented in Table 2 below.

Table 2

Group Number	Ratio of Infected to Total Animals	Percent Protected
1	4/10	60%
2	3/10	70%
3	6/8	25%

5. As illustrated in Table 2, fewer mice immunized with isolated recombinant NGSP polypeptide were found to be infected with *N. gonnorhea* after challenge compared to the unimmunized and unprotected negative controls. The level of protection conferred by NGSP polypeptide of the invention is equivalent to that obtained with the positive control, i.e., inactivated *N. gonnorrhea* whole cell antigen. These results clearly demonstrate that isolated NGSP polypeptide of the present applicati n can confer protection against vaginal

infection by *N. gonnorhea*. These results demonstrate that the isolated NGSP polypeptide is useful to induce a protective immune response against *Neisseria gonorrhea* in a relevant animal model and thus is useful for a protective human vaccine.

I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 12/20/02

Dr. W. James Jackson